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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: PCT/US93/06029</p> <p>(22) International Filing Date: 23 June 1993 (23.06.93)</p> <p>(30) Priority data: 902,935 23 June 1992 (23.06.92) US</p> <p>(60) Parent Application or Grant (63) Related by Continuation US Filed on 07/902,935 (CIP) 23 June 1992 (23.06.92)</p> <p>(71) Applicant (<i>for all designated States except US</i>): DIATECH, INC. [US/US]; 9 Delta Drive, Londonderry, NH 03053 (US).</p>			<p>(72) Inventors; and (75) Inventors/Applicants (<i>for US only</i>) : DEAN, Richard, T. [US/US]; 43 King Road, Bedford, NH 03110 (US). LISTER-JAMES, John [US/US]; 25 Old Stoneway, Bedford, NH 03110 (US).</p> <p>(74) Agent: NOONAN, Kevin, E.; Allegretti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).</p> <p>(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report.</i></p> <p>(88) Date of publication of the international search report: 31 March 1994 (31.03.94)</p>
<p>(54) Title: RADIOACTIVELY-LABELED SOMATOSTANTIN-DERIVED PEPTIDES FOR IMAGING AND THERAPEUTIC USES</p> <p>(57) Abstract</p> <p>This invention relates to therapeutic reagents and peptides, radiodiagnostic reagents and peptides, and methods for producing labeled radiodiagnostic agents. Specifically, the invention relates to peptide derivatives and analogs of somatostatin, and embodiments of such peptides labeled with technetium-99m (Tc-99m), as well as methods and kits for making, radiolabeling and using such peptides to image sites in a mammalian body. The invention also relates to peptide derivatives and analogues of somatostatin labeled with rhenium-186(¹⁸⁶Re) and rhenium-188(¹⁸⁸Re), and methods and kits for making, radiolabeling and using such peptides therapeutically in a mammalian body.</p>			

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 93/06029

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 21-23 and 34-36 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 93/06029

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K7/26 A61K43/00 A61K49/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J.E.RIVIER ET AL 'PEPTIDES; CHEMISTRY, STRUCTURE AND BIOLOGY' 1990, ESCOM, LEIDEN M.F.BEAN ET AL; "Identification of a thioether byproduct in the synthesis of a cyclic disulfide peptide by tandem mass spectrometry" see page 443 - page 445 ---	1, 18
Y	WO,A,90 06949 (SANDOZ) 28 June 1990 see the whole document ---	1-20, 24-33, 37-49
Y	FR,A,2 336 943 (AYESRT, MCKENNA & HARRISON, LTD) 29 July 1977 see the whole document ---	1-20, 24-33, 37-49

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents:

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- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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Date of the actual completion of the international search 15 November 1993	Date of mailing of the international search report 20-12-1993
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentam 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer GROENENDIJK, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 93/06029

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FR,A,2 320 109 (MERCK & CO) 4 March 1977 see the whole document ---	1-20, 24-33, 37-49
Y	US,A,5 095 111 (S.Z.LEVER ET AL) 10 March 1992 see the whole document ---	3-5, 7-17,20, 24-33, 37-49
Y	CHEMICAL ABSTRACTS, vol. 115, no. 15, 14 October 1991, Columbus, Ohio, US; abstract no. 159733, B.LI ET AL 'A NEW BIFUNCTIONAL CHELATING AGENT ALPHA, EPSILON-N,N'-BIS(L-CYTEINYL)-L -LYSINE FOR RADIOLABLEING OF MONOCLONAL ANTIBODIES WITH TC-99M' page 1001 ;column 2 ; see abstract & CHIN. CHEM. LETT. vol. 2, no. 4 , 1991 pages 285 - 288 ---	3-17,20, 24-33, 37-49
Y	WO,A,89 10759 (MALLINCKRODT,INC.) 16 November 1989 see claims 1,2,8-14; example 4 ---	3-5, 7-17,20, 24-33, 37-49
Y	INORGANIC CHEMISTRY vol. 29, no. 16 , 8 August 1990 pages 2948 - 2951 N.BRYSON 'PROTECTING GROUPS IN THE PREPARATION OF THIOLATE COMPLEXES OF TECHNETIUM' see the whole document ---	3-5, 7-17, 24-33, 37-43
Y	EP,A,0 453 082 (HYBRITECH INC.) 23 October 1991 see the whole document ---	43-46
Y	BIOCONJ.CHEM. vol. 2, no. 2 , 1991 , WASHINGTON pages 71 - 76 Y.ARANO ET AL 'A NOVEL BIFUNCTIONAL METABOLISABLE LINKER FOR THE CONJUGATION OF ANTIBODIES WITH RADIONUCLEOTIDES' see the whole document ---	43-46
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 93/06029

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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(30) Priority data: 902,935	23 June 1992 (23.06.92)	US	(74) Agent: NOONAN, Kevin, E.; Allegretti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).
(60) Parent Application or Grant (63) Related by Continuation US Filed on		(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(71) Applicant (for all designated States except US): DIATECH, INC. [US/US]; 9 Delta Drive, Londonderry, NH 03053 (US).		Published <i>Without international search report and to be republished upon receipt of that report.</i>	

(54) Title: RADIOACTIVELY-LABELED SOMATOSTANTIN-DERIVED PEPTIDES FOR IMAGING AND THERAPEUTIC USES

(57) Abstract

This invention relates to therapeutic reagents and peptides, radiodiagnostic reagents and peptides, and methods for producing labeled radiodiagnostic agents. Specifically, the invention relates to peptide derivatives and analogs of somatostatin, and embodiments of such peptides labeled with technetium-99m (Tc-99m), as well as methods and kits for making, radiolabeling and using such peptides to image sites in a mammalian body. The invention also relates to peptide derivatives and analogues of somatostatin labeled with rhenium-186(¹⁸⁶Re) and rhenium-188(¹⁸⁸Re), and methods and kits for making, radiolabeling and using such peptides therapeutically in a mammalian body.

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RADIOACTIVELY-LABELED SOMATOSTATIN-DERIVED PEPTIDES FOR IMAGING AND THERAPEUTIC USES

BACKGROUND OF THE INVENTION

5

1. Field of the Invention

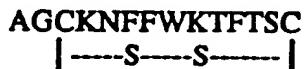
This invention relates to therapeutic reagents and peptides, radiodiagnostic reagents and peptides, and methods for producing labeled radiodiagnostic and radiotherapeutic agents. Specifically, the invention relates 10 to peptide derivatives and analogues of somatostatin, and embodiments of such peptides labeled with technetium-99m (Tc-99m), as well as methods and kits for making, radiolabeling and using such peptides to image sites in a mammalian body. The invention also relates to peptide derivatives and 15 analogues of somatostatin labeled with rhenium-186 (¹⁸⁶Re) and rhenium-188 (¹⁸⁸Re), and methods and kits for making, radiolabeling and using such peptides therapeutically in a mammalian body.

2. Description of the Prior Art

Somatostatin is a tetradecapeptide that is endogenously produced by the 20 hypothalamus and pancreas in humans and other mammals. The peptide has the formula:

Formula I

25



[Single letter abbreviations for amino acids can be found in G. Zubay, Biochemistry (2d ed.), 1988, (MacMillan Publishing: New York), p.33]. This 30 peptide exerts a wide variety of biological effects *in vivo*. It is known to act physiologically on the central nervous system, the hypothalamus, the pancreas, and the gastrointestinal tract.

Somatostatin inhibits the release of insulin and glucagon from the 35 pancreas, inhibits growth hormone release from the hypothalamus, and reduces gastric secretions. Thus, somatostatin has clinical and therapeutic applications for the alleviation of a number of ailments and diseases, both in humans and

other animals. Native somatostatin is of limited utility, however, due to its short half-life *in vivo*, where it is rapidly degraded by peptidases. For this reason, somatostatin analogues having improved *in vivo* stability have been developed in the prior art.

5 Freidinger, U.S. Patent No. 4,235,886 disclose cyclic hexapeptide somatostatin analogues useful in the treatment of a number of diseases in humans.

10 Freidinger, U.S. Patent No. 4,611,054 disclose cyclic hexapeptide somatostatin analogues useful in the treatment of a number of diseases in humans.

Nutt, U.S. Patent No. 4,612,366 disclose cyclic hexapeptide somatostatin analogues useful in the treatment of a number of diseases in humans.

Coy *et al.*, U.S. Patent No. 4,853,371 disclose synthetic octapeptide somatostatin analogues.

15 Coy and Murphy, U.S. Patent No. 4,871,717 disclose synthetic heptapeptide somatostatin analogues.

Coy and Murphy, U.S. Patent No. 4,485,101 disclose synthetic dodecapeptide somatostatin analogues.

20 Coy *et al.*, U.S. Patent No. 4,904,642 disclose synthetic octapeptide somatostatin analogues.

Brady, European Patent Application No. 83111747.8 discloses dicyclic hexapeptide somatostatin analogues useful in the treatment of a number of human diseases.

25 Bauer *et al.*, European Patent Application No. 85810617.2 disclose somatostatin derivatives useful in the treatment of a number of human diseases.

Eck and Moreau, European Patent Application No. 90302760.5 disclose therapeutic octapeptide somatostatin analogues.

Cox, International Patent Application No. PCT/US92/04559 discloses radiolabeled somatostatin derivatives containing two cysteine residues.

30 Somatostatin exerts its effects by binding to specific receptors expressed at the cell surface of cells comprising the central nervous system, the

hypothalamus, the pancreas, and the gastrointestinal tract. These high-affinity somatostatin binding sites have been found to be abundantly expressed at the cell surface of most endocrine-active tumors arising from these tissues. Expression of high-affinity binding sites for somatostatin is a marker for these tumor cells, and specific binding with somatostatin can be exploited to locate and identify tumor cells *in vivo*.

Methods for radiolabeling somatostatin analogues that have been modified so as to contain a tyrosine amino acid (Tyr or Y) are known in the prior art.

Albert *et al.*, UK Patent Application 8927255.3 disclose radioimaging using somatostatin derivatives such as octreotide labeled with ^{123}I .

Bakker *et al.*, J. Nucl. Med. 31: 1501-1509 (1990) describe radioactive iodination of a somatostatin analog and its usefulness in detecting tumors *in vivo*.

Bakker *et al.*, J. Nucl. Med. 32: 1184-1189 (1991) teach the usefulness of radiolabeled somatostatin for radioimaging *in vivo*.

Alternatively, methods for radiolabeling somatostatin by covalently modifying the peptide to contain a radionuclide-chelating group have been disclosed in the prior art.

Albert *et al.*, UK Patent Application 8927255.3 disclose radioimaging using somatostatin derivatives such as octreotide labeled with ^{111}In via a chelating group bound to the amino-terminus.

Albert *et al.*, European Patent Application No. WO 91/01144 disclose radioimaging using radiolabeled peptides related to growth factors, hormones, interferons and cytokines and comprised of a specific recognition peptide covalently linked to a radionuclide chelating group.

Albert *et al.*, European Patent Application No. 92810381.1 disclose somatostatin peptides having amino-terminally linked chelators.

Bodgen and Moreau, International Patent Application Serial No. PCT/US92/01027 disclose compositions and methods for treating proliferative skin disease.

Faglia *et al.*, 1991, J. Clin. Endocrinol. Metab. 73: 850-856 describe the detection of somatostatin receptors in patients.

Kwekkeboom *et al.*, J. Nucl. Med. 32: 981 (1991) Abstract #305 relates to radiolabeling somatostatin analogues with ¹¹¹In.

5 Albert *et al.*, Abstract LM10, 12th American Peptide Symposium: 1991 describe uses for ¹¹¹In-labeled diethylene-triaminopentaacetic acid-derivatized somatostatin analogues.

Krenning *et al.*, 1992, J. Nucl. Med. 33: 652-658 describe clinical scintigraphy using [¹¹¹In][DTPA]octreotide.

10 These methods can be readily adapted to enable detection of tumor cells *in vivo* by radioimaging, based on the expression of high affinity binding sites for somatostatin on tumor cells. Radionuclides which emit high energy gamma radiation can be readily detected by scintigraphy after injection into a human or an animal. A variety of radionuclides are known to be useful for 15 radioimaging, including ⁶⁷Ga, ⁶⁸Ga, ^{99m}Tc (Tc-99m), ¹¹¹In, ¹²³I or ¹²⁵I. The sensitivity of imaging methods using radioactively-labeled peptides is much higher than other techniques known in the art, since the specific binding of the radioactive peptide concentrates the radioactive signal over the cells of interest, for example, tumor cells. This is particularly important for endocrine-active 20 gastrointestinal tumors, which are usually small, slow-growing and difficult to detect by conventional methods. Labeling with technetium-99m (Tc-99m) is advantageous because the nuclear and radioactive properties of this isotope make it an ideal scintigraphic imaging agent. Tc-99m has a single photon energy of 140 keV and a radioactive half-life of about 6 hours, and is readily 25 available from a ⁹⁹Mo-^{99m}Tc generator. Other radionuclides have effective half-lives which are much longer (*for example*, ¹¹¹In, which has a half-life of 60-70 h) or are toxic (*for example*, ¹²⁵I). Although Tc-99m is an ideal radiolabeling reagent, it has not been widely used in the art prior to the present invention [see, *for example*, Lamberts, J. Nucl. Med. 32: 1189-1191 (1991)].

30 Somatostatin and radiolabeled somatostatin analogues can also be used therapeutically. For these applications, the rhenium isotopes ¹⁸⁶Re and ¹⁸⁸Re

are particularly advantageous.

Taylor *et al.*, U.S. Patent No. 5,073,541 disclose a method of treating small cell lung cancer.

Coy and Murphy, International Patent Application Serial No. 5 PCT/US90/07074 disclose somatostatin analogues for therapeutic uses.

Schally *et al.*, European Patent Application Serial No. EPA 911048445.2 disclose cyclic peptides for therapeutic use.

Bomanji *et al.*, 1992, J. Nucl. Med. 33: 1121-1124 describe the use of iodinated (Tyr-3) octreotide for imaging metastatic carcinoid tumors.

10 The use of chelating agents for radiolabeling proteins are known in the prior art, and methods for labeling peptides Tc-99m are disclosed in co-pending U.S. Patent Applications Serial Nos. 07/653,012, 07/757,470, 07/807,062, 07/851,074, 07/871,282, 07/886,752, 07/893,981, 07/955,466, 07/977,628, 08/019,864, 08/044,825 and 08/_____, and PCT International 15 Applications PCT/US92/00757, PCT/US92/10716, PCT/US93/02320, PCT/US93/03687, PCT/US93/04794, and PCT/US93/_____, which are hereby incorporated by reference.

Fritzberg, U.S. Patent No. 4,444,690 describes a series of technetium-chelating agents based on 2,3-bis(mercaptoacetamido) propanoate.

20 Gansow *et al.*, U.S. Patent No. 4,472,509 teach methods of manufacturing and purifying Tc-99m chelate-conjugated monoclonal antibodies.

Reno and Bottino, European Patent Application 87300426.1 disclose radiolabeling antibodies with Tc-99m.

25 Pak *et al.*, European Patent Application No. WO 88/07382 disclose a method for labeling antibodies with Tc-99m.

Rhodes, Sem. Nucl. Med. 4: 281-293 (1974) teach the labeling of human serum albumin with technetium-99m.

Khaw *et al.*, J. Nucl. Med. 23: 1011-1019 (1982) disclose methods for labeling biologically active macromolecules with Tc-99m.

30 Byrne and Tolman, *supra*, disclose a bifunctional thiolactone chelating agent for coupling Tc-99m to biological molecules.

6

Cox *et al.*, Abstract, 7th International Symposium on Radiopharmacology, p. 16, 1991, disclose the use of, Tc-99m-, ¹³¹I- and ¹¹¹In-labeled somatostatin analogues in radiolocalization of endocrine tumors *in vivo* by scintigraphy.

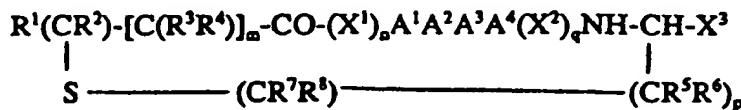
5 Methods for directly labeling somatostatin, derivatives of somatostatin, analogues of somatostatin or peptides that bind to the somatostatin receptor and contain at least 2 cysteine residues that form a disulfide or wherein the disulfide is reduced to the sulfhydryl form, are disclosed in co-pending U.S. Patent Application Serial No. 07/807,062, now U.S. Patent No. 5,225,180, 10 issued July 6, 1993 which is hereby incorporated by reference.

15 There remains a need for synthetic (to make routine manufacture practicable and to ease regulatory acceptance) somatostatin analogues having increased *in vivo* stability, to be used therapeutically and as scintigraphic agents when radiolabeled with Tc-99m for use in imaging tumors *in vivo*. Small synthetic somatostatin analogues are provided by this invention that specifically fulfill this need.

SUMMARY OF THE INVENTION

The present invention provides somatostatin analogues that are peptide reagents for therapeutic and scintigraphic imaging applications. Specifically, the 20 invention provides peptide reagents for preparing scintigraphic imaging agents that are technetium-99m (Tc-99m) labeled. The scintigraphic imaging agents of the invention are comprised of a peptide that is a somatostatin analogue covalently linked to a Tc-99m binding moiety and labeled with Tc-99m. In addition, the invention provides somatostatin analogues that are useful 25 therapeutically, such analogues being radiolabeled with ¹⁸⁶Re and ¹⁸⁸Re.

The somatostatin analogues provided by the invention are somatostatin-receptor binding peptides having the following formula:



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where R^1 and R^2 are independently H, lower alkyl or substituted alkyl, aryl or substituted aryl; R^3 and R^4 are each independently H, lower alkyl or substituted alkyl, aryl or substituted aryl, or either R^3 or R^4 are $N(R^{10})_2$, where each R^{10} is independently H, lower alkyl or a peptide sequence of no more than 10 amino acids, and m is an integer between 0 and 3; X^1 and X^2 are each independently a D- or L- amino acid, and n and q are independently either 0 or 1; A^1 is D- or L-Phe or D- or L-Tyr or 2-naphthylalanine (Nal) or substituted derivatives thereof; A^2 is D- or L-Trp or substituted derivatives thereof; A^3 is D- or L-Lys or homolysine (Hly), 4-amino-cyclohexylalanine (Achxa), 4-aminomethylphenylalanine (Amf), S-(2-aminoethyl)cysteine (Aec), S-(3-aminopropyl)cysteine (Apc), O-(2-aminoethyl) serine (Aes), O-(3-aminopropyl)serine (Aps) or substituted derivatives thereof; A^4 is Thr, Ser, Val, Phe, Leu, Ile or 2-amino-isobutyric acid (Aib), 2-aminobutyric acid (Abu), norvaline (Nva), or norleucine (Nle), most preferably Thr or Val; X^3 is H, -COOR⁹, -CH₂OH, CH₂COOR⁹, or -CON(R⁹)₂, where each R^9 is independently H, lower linear or cyclic alkyl or substituted derivatives thereof, or a peptide having an amino acid sequence of no more than 10 residues; R^5 and R^6 are each independently H or lower alkyl and p is either 0, 1 or 2; and R^7 and R^8 are independently H, lower alkyl or substituted lower alkyl, or either R^7 and R^8 are -COOH or a derivative thereof. In a preferred embodiment, A^1 is Phe or Tyr, A^2 is Trp or most preferably D-Trp, A^3 is Lys and A^4 is Thr or Val.

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In a first aspect of the present invention are provided peptide reagents that are somatostatin analogues as described herein having increased *in vivo* stability compared with native somatostatin, and that are therapeutically useful in the alleviation of diseases or other ailments in humans or other animals.

The invention also provides pharmaceutical compositions comprising the somatostatin receptor-binding peptides of the invention in a pharmaceutically

acceptable carrier.

The invention also provides a method for alleviating somatostatin-related diseases in animals, preferably humans, comprising administering a therapeutically effective amount of the somatostatin analogues of the invention to the animal. In preferred embodiments, the amount of the somatostatin analogue administered is from about 0.1 to about 50 mg/kg body weight/day.

Another aspect of the present invention provides reagents for preparing scintigraphic imaging agents, each reagent comprising a peptide that is somatostatin analogue and is covalently linked to a Tc-99m binding moiety.

It is an advantage of the somatostatin analogues provided by this invention that the thioether linkage contained therein is stable under the conditions of Tc-99m conjugation to the covalently linked Tc-99m binding moiety. In contrast, Tc-99m conjugation to a Tc-99m binding moiety covalently linked to native somatostatin, or to a somatostatin analogue having a disulfide bond, can result in reduction of the disulfide accompanied by a loss of biological activity. Such loss of biological activity can also occur *in vivo* using native somatostatin, or to any somatostatin analogue having a disulfide bond. The present invention is not subject to similar losses in biological activity *in vivo* because the thioether linkage in each of the somatostatin analogues of the invention is a stable covalent bond.

It is another advantage of the somatostatin analogues provided by this invention that the covalent linkage between the amino terminus and the cysteine protecting moiety acts to protect the peptide from degradation by exopeptidases.

A first aspect of the reagents provided by the invention for preparing scintigraphic imaging agents of the invention are reagents, each comprised of a peptide that is a somatostatin analogue that is covalently linked to a Tc-99m binding moiety having formula:



wherein $(\text{pgp})^s$ is H or a thiol protecting group and (aa) is an amino acid. In a preferred embodiment, the amino acid is glycine.

In a second embodiment, the invention provides peptide reagents capable of being Tc-99m labeled for imaging sites within a mammalian body, each comprising a somatostatin analogue that is covalently linked to a Tc-99m binding moiety of formula:

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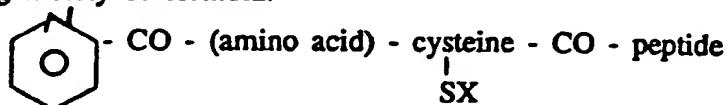
II.



wherein A is H, HOOC, H₂NOC, (peptide)-NHOC, (peptide)-OOC or R'''; B is H, SH or -NHR''', -N(R''')-(peptide) or R''''; X is SH or -NHR''', -N(R''')-(peptide) or R''''; R', R'', R''' and R'''' are independently H or straight or branched chain or cyclic lower alkyl; n is 0, 1 or 2; and: (1) where B is -NHR''' or -N(R''')-(peptide), X is SH and n is 1 or 2; (2) where X is -NHR''' or -N(R''')-(peptide), B is SH and n is 1 or 2; (3) where B is H or R''''', A is HOOC, H₂NOC, (peptide)-NHOC, (peptide)-OOC, X is SH and n is 0 or 1; (4) where A is H or R''''', then where B is SH, X is -NHR''' or -N(R''')-(peptide) and where X is SH, B is -NHR''' or -N(R''')-(peptide); (5) where X is H or R''''', A is HOOC, H₂NOC, (peptide)-NHOC, (peptide)-OOC and B is SH; (6) where Z is methyl, X is methyl, A is HOOC, H₂NOC, (peptide)-NHOC, (peptide)-OOC and B is SH and n is 0; and (7) where Z is SH and X is SH, n is not 0; and wherein the thiol moiety is in the reduced form.

In another embodiment, the invention provides peptide reagents capable of being labeled with Tc-99m for imaging sites within a mammalian body, each comprising a somatostatin analogue that is covalently linked to a Tc-99m binding moiety of formula:

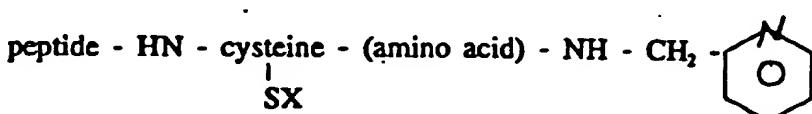
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[for purposes of this invention, radiolabel-binding moieties having this structure will be referred to as picolinic acid (Pic)-based moieties]

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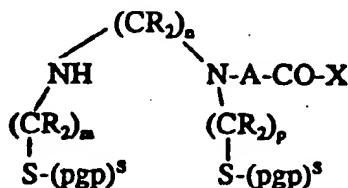


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wherein X is H or a protecting group; (amino acid) is any amino acid and the radiolabel-binding moiety is covalently linked to the peptide. For purposes of this invention, radiolabel-binding moieties having this structure will be referred to as picolylamine (Pica)-based moieties. In a preferred embodiment, the 5 amino acid is glycine and X is an acetamidomethyl protecting group.

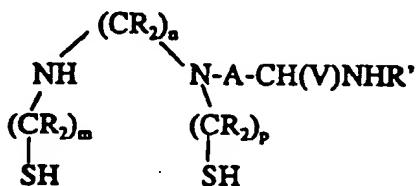
Yet another embodiment of the invention provides peptide reagents capable of being labeled with Tc-99m for imaging sites within a mammalian body, each comprising a somatostatin analogue that is covalently linked to a Tc-99m binding moiety that is a bisamino bisthiol Tc-99m binding moiety. 10 The bisamino bisthiol Tc-99m binding moiety in this embodiment of the invention has the formula:

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wherein each R can be independently H, CH, or C₂H₅; each (pgp)^s can be independently a thiol protecting group or H; m, n and p are independently 2 or 3; A is linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof; and X is peptide; or



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wherein each R is independently H, CH, or C₂H₅; m, n and p are independently 2 or 3; A is linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof; V is H or CO-peptide; R' is H or peptide; provided that when V is H, R' is peptide and when R' is H, V is peptide. For purposes of this invention, radiolabel-binding moieties having these structures will be referred to as "BAT" moieties.

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The invention also comprises scintigraphic imaging agents that are complexes of the peptide reagents of the invention with Tc-99m and methods

for radiolabeling the peptide reagents of the invention with Tc-99m. Radiolabeled complexes provided by the invention are formed by reacting the peptide reagents of the invention with Tc-99m in the presence of a reducing agent. Preferred reducing agents include but are not limited to dithionite ion, stannous ion and ferrous ion. Complexes of the invention are also formed by labeling the peptide reagents of the invention with Tc-99m by ligand exchange of a prereduced Tc-99m complex as provided herein.

The invention also provides kits for preparing scintigraphic imaging agents that are the peptide reagents of the invention radiolabeled with Tc-99m. Kits for labeling the peptide reagents of the invention with Tc-99m are comprised of a sealed vial containing a predetermined quantity of a peptide reagent of the invention and a sufficient amount of reducing agent to label the peptide with Tc-99m.

This invention provides methods for preparing peptide reagents of the invention by chemical synthesis *in vitro*. In a preferred embodiment, peptides are synthesized by solid phase peptide synthesis.

This invention provides methods for using scintigraphic imaging agents that are Tc-99m labeled peptide reagents for imaging sites within a mammalian body by obtaining *in vivo* gamma scintigraphic images. These methods comprise administering an effective diagnostic amount of Tc-99m labeled peptide reagents of the invention and detecting the gamma radiation emitted by the Tc-99m label localized at the site within the mammalian body.

This invention provides reagents for preparing a radiolabeled somatostatin receptor-binding agent comprising the somatostatin receptor-binding peptides of the invention covalently linked to a radiolabel-binding moiety. In a preferred embodiment, the reagent is radioactively labeled with Tc-99m. In another preferred embodiment, the reagent is radioactively labeled with ¹¹¹Re or ¹⁸⁶Re.

The invention also provides methods for alleviating somatostatin-related diseases in animals, preferably humans, comprising administering a therapeutically effective amount of the radiolabeled somatostatin-binding peptide reagents of the invention to the animal. In preferred embodiments, the reagent

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is radioactively labeled with ^{116}Re or ^{188}Re .

The reagents of the invention may also be comprised of a polyvalent linking moiety. Polyvalent linking moieties of the invention are comprised of at least 2 identical linker functional groups capable of covalently bonding to somatostatin analogue peptides or Tc-99m binding moieties. Preferred linker functional groups are primary or secondary amines, hydroxyl groups, carboxylic acid groups or thiol-reactive groups. In preferred embodiments, the polyvalent linking moieties are comprised of *bis*-succinimidylmethylether (BSME), 4-(2,2-dimethylacetyl)benzoic acid (DMBA), *N*-[2-(*N,N*'-*bis*(2-succinimidethyl)aminoethyl)]-*N⁸,N⁹*-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide (BAT-BS), *tris*(succinimidylethyl)amine (TSEA), *bis*-succinimidohexane (BSH), 4-(O-CH₂CO-Gly-Gly-Cys.amide)acetophenone (ETAC) or a derivative thereof.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides peptide reagents for preparing radiolabeled imaging agents for imaging site within a mammalian body. The peptide reagents of the invention each comprise a somatostatin analogue that is covalently linked to a Tc-99m binding moiety. The invention also provides somatostatin analogues having an increased *in vivo* stability and that are useful for alleviating diseases or other ailments in humans or other animals.

The invention provides a method for using the somatostatin analogues of the invention to alleviate diseases or other ailments in animals, preferably humans. These diseases and ailments include but are not limited to diabetes and diabetes-related retinopathy, cirrhosis of the liver and hepatitis infection, bleeding ulcers and other gastrointestinal bleeding, pancreatitis, central nervous system disorders, endocrine disorders, Alzheimer's disease, acromegaly and other diseases and disorders related to the production of inappropriate levels of growth hormone *in vivo*, and cancer, particularly those cancers whose growth

is dependent or influenced by growth hormone production. Dosages of the somatostatin analogues provided by the invention may be the same as those dosages of native somatostatin routinely used for treatment of the above or other diseases, or less of the compounds of the invention may be administered due to their longer *in vivo* half-life.

Labeling with Tc-99m is an advantage of the present invention because the nuclear and radioactive properties of this isotope make it an ideal scintigraphic imaging agent. This isotope has a single photon energy of 140 keV and a radioactive half-life of about 6 hours, and is readily available from a ⁹⁹Mo-^{99m}Tc generator. Other radionuclides known in the prior art have effective half-lives which are much longer (*for example*, ¹¹¹In, which has a half-life of 67.4 h) or are toxic (*for example*, ¹²⁵I).

Radiotherapeutic embodiments of the invention, on the other hand, are advantageously labeled with ¹⁸⁶Re or ¹⁸⁸Re. Such embodiments are useful in the treatment of somatostatin-related diseases or other ailments in animals, preferably humans, including but not limited to cancer and other diseases characterized by the growth of malignant or benign tumors capable of binding somatostatin or somatostatin analogues *via* the expression of somatostatin receptors on the cell surface of cells comprising such tumors.

In the Tc-99m binding moieties and peptides covalently linked to such moieties that contain a thiol covalently linked to a thiol protecting groups [(pgp)ⁿ] provided by the invention, the thiol-protecting groups may be the same or different and may be but are not limited to:

- CH₂-aryl (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
- CH-(aryl)₂, (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
- C-(aryl)₃, (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
- CH₂-(4-methoxyphenyl);
- CH-(4-pyridyl)(phenyl)₂;
- C(CH₃)₃;
- 9-phenylfluorenyl;
- CH₂NHCOR (R is unsubstituted or substituted alkyl or aryl);

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- CH₂-NHCOOR (R is unsubstituted or substituted alkyl or aryl);
- CONHR (R is unsubstituted or substituted alkyl or aryl);
- CH₂-S-CH₂-phenyl

Preferred protecting groups have the formula -CH₂-NHCOR wherein R 5 is a lower alkyl having 1 and 8 carbon atoms, phenyl or phenyl-substituted with lower alkyl, hydroxyl, lower alkoxy, carboxy, or lower alkoxy carbonyl. The most preferred protecting group is an acetamidomethyl group.

Each somatostatin receptor-binding peptide-containing embodiment of the invention is comprised of a sequence of amino acids. The term amino acid as 10 used in this invention is intended to include all L- and D- amino acids, naturally occurring and otherwise. Reagents comprising somatostatin receptor-binding peptides provided by the invention include but are not limited to the following illustrative examples of the peptide embodiments of the invention:

15 CH₂CO.YW_DKTCTC_{Acm}.GC_{Acm}.amide
 CH₂CO.YW_DKTC

20 CH₂CO.YW_DKTC.amide
 CH₂CO.YW_DKTCT

25 CH₂CO.YW_DKTCT(CH₂OH)
 CH₂CO.YW_DKTCTGGC_{Mob}.amide
 CH₂CO.FFW_DKTFC

30 CH₂CO.FFW_DKTFC.[BAM]
 CH₂CO.FFW_DKTFC.amide
 CH₂CO.FW_DKT.Hcy

35 CH₂CO.FW_DKTCD
 CH₂CO.FW_DKT.Hcy.amide
 CH₂CO.FW_DKT.Pen

40 CH₂CO.NFFW_DKTFTC

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CH.CO.FFW_DKTFCC_{Acm}GC_{Acm}.amideCH.CO.FFW_DKTF.Hcy5 PbCH₂CHCO.YW_DKTCCH.CO.YW_DKT.Hhc10 CH.CO.YW_DKT.Hhc.amideCH.CO.FFW_DKTF.HhcCH.CO.FYW_DKTFC

As used herein, the following amino acids and amino acid analogues are intended to be represented by the following abbreviations: Hcy is homocysteine, prepared by alkaline hydrolysis of L-homocysteine lactone; Hhc is homohomocysteine; Pen is penicillamine; Mob is the sulphydryl protecting group 4-methoxybenzyl; Acm is the sulphydryl protecting group acetamidomethyl; [BAM] is (*N,N*'-bis(2-mercapto-2-methylpropyl)-1,4,10-triazadecane; Aib is aminoisobutyric acid; Nal is 2-naphthylalanine; Ain is 2-aminoindanoic acid; Hly is homolysine; Achxa is 4-amino-cyclohexylalanine; Amf is 4-aminomethylphenylalanine; Aec is S-(2-aminoethyl)cysteine; Apc is S-(3-aminopropyl)cysteine; Aes is O-(2-aminoethyl)serine; Aps is O-(3-aminopropyl)serine; Abu is 2-aminobutyric acid; Nva is norvaline; Aca is 6-aminocaproic acid; and Nle is norleucine. All naturally-occurring amino acids are abbreviated using standard abbreviations (which can be found in G. Zubay, *Biochemistry* (2d. ed.), 1988 (MacMillen Publishing: New York) p.33. T(CH₂OH) represents a threoninol residue, wherein the carboxyl group of the amino acid is reduced to a primary alcohol, incorporated into the peptide using the procedure of Neugebauer *et al.* (1990, Peptides: Proceedings of the 11th American Peptide Symposium, pp. 1020-21).

It will also be understood by those with skill in the art that the convention of representing by underlining a covalent bond between the sidechain sulfur atom of a cysteine residue or derivative thereof and a protecting group or other residue is used herein.

Somatostatin analogue peptides of the present invention can be chemically synthesized *in vitro*. Peptides of the present invention can generally advantageously be prepared on a peptide synthesizer. The peptides of this invention can be synthesized wherein the radiolabel-binding moiety is covalently linked to the peptide during chemical synthesis *in vitro*, using techniques well known to those with skill in the art. Such peptides covalently-linked to the radiolabel-binding moiety during synthesis are advantageous because specific sites of covalent linkage can be determined.

Radiolabel binding moieties of the invention may be introduced into the target somatostatin analogue peptides during peptide synthesis. For embodiments comprising picolinic acid [(Pic-); e.g., Pic-Gly-Cys(protecting group)-], the radiolabel-binding moiety can be synthesized as the last (i.e., amino-terminal) residue in the synthesis. In addition, the picolinic acid-containing radiolabel-binding moiety may be covalently linked to the ϵ -amino group of lysine to give, for example, α N(Fmoc)-Lys- ϵ N[Pic-Gly-Cys(protecting group)], which may be incorporated at any appropriate position in the peptide chain. This sequence is particularly advantageous as it affords an easy mode of incorporation into the target somatostatin analogue peptide.

Similarly, the picolylamine (Pica)-containing radiolabel-binding moiety [-Cys(protecting group)-Gly-Pica] can be prepared during peptide synthesis by including the sequence [-Cys(protecting group)-Gly-] at the carboxyl terminus of the peptide chain. Following cleavage of the peptide from the resin the carboxyl terminus of the peptide is activated and coupled to picolylamine. This synthetic route requires that reactive side-chain functionalities remain masked (protected) and do not react during the conjugation of the picolylamine.

This invention also provides small synthetic peptides that are somatostatin analogues and incorporate bisamine bishiol (BAT) chelators that may be labeled with Tc-99m.

This invention provides for the incorporation of these chelators into virtually any position in the peptide, via covalently linkage to any appropriate functional group of the peptide, except that the chelating moieties of the

invention are not covalently linked to functional groups comprising the amino acid side chains of the amino acids A¹, A², A³ or A⁴.

In forming a complex of radioactive technetium with the reagents of this invention, the technetium complex, preferably a salt of Tc-99m pertechnetate, is reacted with the reagent in the presence of a reducing agent. Preferred reducing agents are dithionite, stannous and ferrous ions; the most preferred reducing agent is stannous chloride. Means for preparing such complexes are conveniently provided in a kit form comprising a sealed vial containing a predetermined quantity of a reagent of the invention to be labeled and a sufficient amount of reducing agent to label the reagent with Tc-99m. Alternatively, the complex may be formed by reacting a reagent of this invention with a pre-formed labile complex of technetium and another compound known as a transfer ligand. This process is known as ligand exchange and is well known to those skilled in the art. The labile complex may be formed using such transfer ligands as tartrate, citrate, gluconate or mannitol, for example. Among the Tc-99m pertechnetate salts useful with the present invention are included the alkali metal salts such as the sodium salt, or ammonium salts or lower alkyl ammonium salts.

In a preferred embodiment of the invention, a kit for preparing technetium-labeled peptides is provided. An appropriate amount of the peptide reagent is introduced into a vial containing a reducing agent, such as stannous chloride, in an amount sufficient to label the peptide with Tc-99m. An appropriate amount of a transfer ligand as described (such as tartrate, citrate, gluconate or mannitol, for example) can also be included. The kit may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. The components of the kit may be in liquid, frozen or dry form. In a preferred embodiment, kit components are provided in lyophilized form.

Radiolabeled imaging reagents according to the present invention may be prepared by the addition of an appropriate amount of Tc-99m or Tc-99m

complex into the vials and reaction under conditions described in Example 2 hereinbelow.

Radioactively-labeled scintigraphic imaging agents provided by the present invention are provided having a suitable amount of radioactivity. In forming Tc-99m radioactive complexes, it is generally preferred to form radioactive complexes in solutions containing radioactivity at concentrations of from about 0.01 millicurie (mCi) to 100 mCi per mL.

The imaging reagents provided by the present invention can be used for visualizing organs such as the kidney for diagnosing disorders in these organs, and tumors, in particular gastrointestinal tumors, myelomas, small cell lung carcinoma and other APUDomas, endocrine tumors such as medullary thyroid carcinomas and pituitary tumors, brain tumors such as meningiomas and astrocytomas, and tumors of the prostate, breast, colon, and ovaries can also be imaged. In accordance with this invention, the Tc-99m labeled peptide reagents are administered in a single unit injectable dose. The Tc-99m labeled peptide reagents provided by the invention may be administered intravenously in any conventional medium for intravenous injection such as an aqueous saline medium, or in blood plasma medium. Generally, the unit dose to be administered has a radioactivity of about 0.01 mCi to about 100 mCi, preferably 1 mCi to 20 mCi. The solution to be injected at unit dosage is from about 0.01 mL to about 10 mL. After intravenous administration, imaging *in vivo* can take place in a matter of a few minutes. However, imaging can take place, if desired, in hours or even longer, after the radiolabeled peptide is injected into a patient. In most instances, a sufficient amount of the administered dose will accumulate in the area to be imaged within about 0.1 of an hour to permit the taking of scintiphotos. Any conventional method of scintigraphic imaging for diagnostic purposes can be utilized in accordance with this invention.

The somatostatin receptor-binding peptides of the invention may be used clinically to promote regression of certain types of tumors, particularly those that express somatostatin receptors. The somatostatin analogue peptides of the

invention can also be used to reduce the hormonal hypersecretion that often accompanies certain cancers, such as the APUDomas. Peptides of the invention used as therapeutic agents may be administered by any appropriate route, including intravenous, intramuscular or by mouth, and in any acceptable pharmaceutical carrier, in doses ranging from about 0.1 to about 49 mg/kg body weight/day.

This invention also provides peptides radiolabeled with rhenium-186 or rhenium-188 that may be used for radiotherapy of certain tumors as described above. For this purpose, an amount of radioactive isotope from about 10mCi to about 200mCi may be administered *via* any suitable clinical route, preferably by intravenous injection.

The methods for making and labeling these compounds are more fully illustrated in the following Examples. These Examples illustrate certain aspects of the above-described method and advantageous results, and are shown by way of illustration and not limitation.

EXAMPLE 1

Solid Phase Peptide Synthesis

Solid phase peptide synthesis (SPPS) was carried out on a 0.25 millimole (mmole) scale using an Applied Biosystems Model 431A Peptide Synthesizer and using 9-fluorenylmethyloxycarbonyl (Fmoc) amino-terminus protection, coupling with dicyclohexylcarbodiimide/hydroxybenzotriazole or 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/hydroxybenzotriazole (HBTU/HOBT), and using *p*-hydroxymethylphenoxy-methylpolystyrene (HMP) resin for carboxyl-terminus acids or Rink amide resin for carboxyl-terminus amides.

Where appropriate, the following amino acid derivatives were synthesized. Homocysteine was prepared by alkaline hydrolysis of L-homocysteine lactone. Threoninol residues, wherein the carboxyl group of the amino acid is reduced to a primary alcohol, are introduced into the peptides of the invention where appropriate using the procedure of Neugebauer *et al.*

(1990, Peptides: Proceedings of the 11th American Peptide Symposium, pp. 1020-21). Fmoc.Hcy(Trt) and Fmoc.Pen(Trt) were prepared from the appropriate amino acids by tritylation with triphenylmethanol in TFA, followed by Fmoc derivitization as described by Atherton *et al.* (1989, Solid Phase Peptide Synthesis, IRL Press: Oxford). Fmoc.homohomo-cysteine(Trt) was prepared by reducing *N,N*-bis-Boc-glutamic acid- α -methyl ester with borane-THF, followed by mesylation and reaction with trityl-mercaptide, followed by removal of the Boc groups with BF₃OEt in acetic acid, and then Fmoc derivitization as described above. PhCH₂CHBrCOOH was prepared by treating phenylalanine (in a solution of water and TFA/ saturated with NaBr) with sodium nitrate, followed by distillation to recover the pure product.

Where appropriate, 2-chloroacetyl, 2-bromoacetyl and 2-bromo-3-phenylpropionyl groups were introduced either by using the appropriate 2-halo acid as the last residue coupled during SPPS, or by treating the N-terminus free amino acid peptide bound to the resin with either 2-halo acid/diisopropylcarbodiimide/*N*-hydroxysuccinimide/NMP or 2-halo acid anhydride/diisopropylethylamine/NMP.

Where appropriate, HPLC-purified 2-haloacylated peptides were cyclized by stirring an 0.1-1.0 mg/mL solution in phosphate or bicarbonate buffer or dilute ammonium hydroxide (pH 8.0), optionally containing 0.5-1.0 mM EDTA, or acetonitrile or THF for 1-48 h followed optionally by acidification with acetic acid, lyophilization and HPLC purification.

Where appropriate, [BAM] (*N¹,N⁴*-bis(2-mercapto-2-methylpropyl)-1,4,10-triazadecane) was conjugated to the peptide by first activating the peptide carboxylate with a mixture of diisopropylcarbodiimide/ *N*-hydroxysuccinimide or HBTU/HOBt in DMF, NMP or methylene chloride, followed by coupling in the presence of diisopropylethylamine. After coupling, the conjugates were deprotected as described above.

Where appropriate, BSME adducts were prepared by reacting single thiol-containing peptides (5 to 50 mg/mL in DMF buffered to pH 7 with *N*-methyl-morpholine or *N*-ethyl-morpholine, or 50mM sodium phosphate buffer,

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pH 7-8, optionally containing 0.5mM EDTA or DMF or THF or acetonitrile) with 0.5 molar equivalents of BMME (*bis*-maleimidomethylether) pre-dissolved in acetonitrile at room temperature for approximately 1-18 hours. The solution was concentrated and the product was purified by HPLC.

5 Where appropriate, TSEA adducts were prepared by reacting single thiol-containing peptide (at concentrations of 10 to 100 mg/mL peptide in DMF buffered to pH 7 with N-methyl-morpholine or N-ethyl-morpholine, or 5 to 50 mg/mL peptide in 50mM sodium phosphate, pH 7-8, optionally containing 0.5mM EDTA or DMF or THF or acetonitrile) with 0.33 molar equivalents of TMEA (*tris*(2-maleimidooethyl)amine) pre-dissolved in acetonitrile or DMF, with or without 1 molar equivalent of triethanolamine, at room temperature for approximately 1-18h. Such reaction mixtures containing adducts were concentrated and the adducts were then purified using HPLC.

10 Where appropriate, BAT-BS (*N*-[2-(*N',N'*-*bis*(2-succinimidooethyl)aminoethyl])-*N⁶,N⁹*-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide adducts were prepared by reacting single thiol-containing peptide (at concentrations of 2 to 50 mg/mL peptide in DMF buffered to pH 7 with N-methyl-morpholine or N-ethyl-morpholine, or in 50mM sodium phosphate (pH 7-8), optionally containing 0.5mM EDTA or DMF or THF or acetonitrile) with 0.5 molar equivalents of BAT-BM (*N*-[2-(*N',N'*-*bis*(2-maleimidooethyl)aminoethyl])-*N*'-(*t*-butoxycarbonyl)-*N⁶,N⁹*-*bis*(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanamide) pre-dissolved in acetonitrile or THF, at room temperature for approximately 1-18h. The solution was then evaporated to dryness and [BAT-BS]-peptide conjugates deprotected by treatment with 10mL TFA and 0.2mL triethylsilane for 1h. The solution was concentrated, the product adducts precipitated with ether, and then purified by HPLC.

15 Resin-bound products were routinely cleaved using a solution of trifluoroacetic acid or trifluoroacetic acid and methylene chloride, or optionally a solution of trifluoroacetic acid, water, thioanisole, ethanedithiol, and triethylsilane, prepared in ratios of 100 : 5 : 5 : 2.5 : 2 for 0.5 - 3 h at room temperature. Crude peptides were purified by preparative high pressure liquid

chromatography (HPLC) using a Waters Delta Pak C18 column and gradient elution using 0.1% trifluoroacetic acid (TFA) in water modified with acetonitrile. Acetonitrile was evaporated from the eluted fractions which were then lyophilized. The identity of each product was confirmed by fast atom bombardment mass spectroscopy (FABMS) or by electrospray mass spectroscopy (ESMS).

The following somatostatin analogues were synthesized as provided herein, and the products of such synthesis identified by FABMS (MH^+ values in parentheses):

10	<u>CH₃CO.YW_DKTCTC_{Acm}GC_{Acm}.amide</u>	(1246)
15	<u>CH₃CO.YW_DKTC</u>	(740)
	<u>CH₃CO.YW_DKTC.amide</u>	(740)
	<u>CH₃CO.YW_DKTCT</u>	(841)
20	<u>CH₃CO.YW_DKTCT(CH₃OH)</u>	(828)
	<u>CH₃CO.YW_DKTCTGGC_{Mab}.amide</u>	(1178)
25	<u>CH₃CO.FFW_DKTFC</u>	(1018)
	<u>CH₃CO.FFW_DKTFC.[BAM]</u>	(1322)
	<u>CH₃CO.FFW_DKTFC.amide</u>	(1017)
30	<u>CH₃CO.FW_DKT.Hcy</u>	(738)
	<u>CH₃CO.FW_DKTC_D</u>	(724)
35	<u>CH₃CO.FW_DKT.Hcy.amide</u>	(737)
	<u>CH₃CO.FW_DKT.Pen</u>	(752)
	<u>CH₃CO.NFFW_DKTFC</u>	(1234)
40	<u>CH₃CO.FFW_DKTFCC_{Acm}GC_{Acm}.amide</u>	(1422)
	<u>CH₃CO.FFW_DKTF.Hcy</u>	(1032)

23

	<u>PhCH₂CHCO.YW_bKTC</u>	(830)
	<u>CH₂CO.YW_bKT.Hhc</u>	(769)
5	<u>CH₂CO.YW_bKT.Hhc.amide</u>	(768)
	<u>CH₂CO.FFW_bKTF.Hhc</u>	(1046)
	<u>CH₂CO.FYW_bKTFC</u>	(1033)

10

EXAMPLE 2**A General Method for Radiolabeling with Tc-99m**

0.1 mg of a peptide prepared as in Example 2 was dissolved in 0.1 mL of water or 50/50 ethanol/water or phosphate-buffered saline or 50 mM potassium phosphate buffer (pH = 5, 6 or 7.4). Tc-99m gluceptate was prepared by reconstituting a Glucoscan vial (E.I. DuPont de Nemours, Inc.) with 1.0 mL of Tc-99m sodium pertechnetate containing up to 200 mCi and allowed to stand for 15 minutes at room temperature. 25 μ l of Tc-99m gluceptate was then added to the peptide and the reaction allowed to proceed at room temperature or at 100°C for 15-30 min and then filtered through a 0.2 μ m filter.

The Tc-99m labeled peptide purity was determined by HPLC using the following conditions: a Waters Delta Pak RP-18, 5 μ , 4.6mm x 220mm analytical column was loaded with each radiolabeled peptide, and the peptides eluted at a solvent flow rate equal to 1 mL/min. Gradient elution was performed beginning with 100% solvent A (0.1% CF₃COOH/H₂O) and ending with 100% solvent B (0.1% CF₃COOH/90% CH₃CN/H₂O) over the course of 10-20 min.

Radioactive components were detected using an in-line radiometric detector linked to an integrating recorder. Tc-99m gluceptate and Tc-99m sodium pertechnetate elute between 1 and 4 minutes under these conditions, whereas the Tc-99m labeled peptides eluted after a much greater amount of time, as illustrated in Table I below.

TABLE I

<u>Peptides</u>	FABMS <u>MH⁺</u>	Radiochemical Yield	HPLC R(min)
P389	1422	99%*	15.1-16.9
P428	1322	99%**	18.8

CH₂COFFW_nKTFCC_{A_{con}}GC_{A_{con}}.amide = P389

CH₂COFFW_nKTFC.[BAM] = P428

10

* 1:1 ethanol:water, 100°C

** 1:1 ethanol:water, room temperature

15

EXAMPLE 3

Inhibition of Binding of [¹²⁵I-Tyr¹¹]somatostatin-14 to AR42J Rat Pancreatic Tumor Cell Membranes

The ability of various somatostatin analogues of the invention to bind to somatostatin receptors *in vitro* was demonstrated by assaying the ability of such analogues to inhibit binding of a radiolabeled somatostatin analogue to somatostatin receptor-containing cell membranes. The rat pancreatic tumor cell line AR42J which expresses the somatostatin receptor was cultured in Dulbecco's minimal essential media (DMEM) supplemented with 10% fetal bovine serum (FBS) and 8mM glutamine in a humidified 5% CO₂ atmosphere at 37°C in T-flasks. Harvested cells were homogenized in cold 50mM Tris-HCl buffer (pH 7.4) and the homogenate then centrifuged at 39,000g for 10min at 4°C. Pellets were washed once with buffer and then resuspended in an ice-cold solution of 10mM Tris-HCl (pH 7.4). Equal aliquots of this cell membrane preparation were incubated with [¹²⁵I-Tyr¹¹]somatostatin-14 (at a final concentration of 0.5nM and 750,000cpm/mL, at a specific activity of 2000Ci/mmol, Amersham, Arlington Heights, IL) and peptide at a final concentration of from 10⁻¹¹M to 10⁻⁶M in a solution of 50mM HEPES (pH 7.4) containing 1% bovine serum albumin (BSA), 5mM MgCl₂, Trasylol (200,000 International Units), bacitracin (0.02mg/mL) and phenylmethylsulfonylfluoride

25

(0.02mg/mL) for 25min at 30°C. Using a filtration manifold, this mixture was filtered through a polyethyleneimine-washed GC/F filter (Whatman, Maidstone, England), and the residue remaining on the filter washed thrice with 5mL cold HEPES buffer. The filter and a sample of the filter washings were then counted in a gamma counter. To assess non-specific binding, the assay was performed in the presence of unlabeled somatostatin-14 at 200nM. Data analysis including Hill plots of the data provided inhibition constants (see Bylund & Yamamura, "Methods of receptor binding", in Methods in Neurotransmitter Receptor Analysis, Yamamura *et al.*, eds., Raven Press: New York, 1990).

These results are presented in the following Table. The data show that the peptides of the instant invention have a high affinity of binding for somatostatin receptors.

15

TABLE II

Peptides	K_i (nM)
<u>CH₃CO.FYW_DKTFC</u>	0.16
<u>CH₃CO.FFW_DKTF.Hhc</u>	0.41
<u>CH₃CO.FFW_DKTFC.amide</u>	0.45
<u>CH₃CO.FFW_DKTFC.[BAM]</u>	1.9
<u>CH₃CO.NFFW_DKTFTC</u>	2.7
<u>CH₃CO.FFW_DKTFC</u>	4.0
<u>CH₃CO.FFW_DKTFCC_{Acm}GC_{Acm}.amide</u>	7.5
<u>CH₃CO.FFW_DKTF.Hcy</u>	9.8

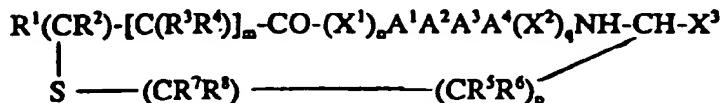
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It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

What is claimed is:

1. A somatostatin receptor-binding peptide having the formula:

5



10 wherein R^1 and R^2 are independently H, lower alkyl or substituted alkyl, aryl or substituted aryl;

15 R^3 and R^4 are each independently H, lower alkyl or substituted alkyl, aryl or substituted aryl, or wherein either R^3 or R^4 is $N(R^{10})_2$, where each R^{10} is independently H, lower alkyl or a peptide sequence of no more than 10 amino acids, and m is an integer between 0 and 3;

20 X^1 and X^2 are each independently a D- or L- amino acid, and n and q are independently either 0 or 1;

25 A^1 is D- or L-Phe or D- or L-Tyr or Nal or substituted derivatives thereof;

A^2 is D- or L-Trp or substituted derivatives thereof;

A^3 is D- or L-Lys or Hly, Achxa, Amf, Aec, Apc, Aes, Aps or substituted derivatives thereof;

A^4 is Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva or Aib;

X^3 is H, -COOR', -CH₂OH, CH₂COOR', or -CON(R')₂, where each R' is independently H, lower linear or cyclic alkyl or substituted derivatives thereof, or a peptide having an amino acid sequence of no more than 10 residues;

30 R^5 and R^6 are each independently H or lower alkyl and p is either 0, 1 or 2;

 and R^7 and R^8 are independently H, lower alkyl or substituted lower alkyl, or either R^7 or R^8 are -COOH or a derivative thereof.

2. A pharmaceutical composition comprising the somatostatin receptor-binding peptide of Claim 1 in a pharmaceutically acceptable carrier.

3. A reagent for preparing a scintigraphic imaging agent for imaging sites within a mammalian body comprising the somatostatin receptor-binding peptide of Claim 1 and a technetium-99m binding moiety covalently linked thereto, the technetium-99m binding moiety having the formula:

5

I.



wherein $(pg)^s$ is H or a thiol protecting group and (aa) is an amino acid;

II.



10

wherein A is H, HOOC, H_2NOC , (peptide)-NHOC, (peptide)-OOC or R'''' ;

15

B is H, SH, -NHR'', -N(R'')-(peptide), or R'''' ;

X is H, SH, -NHR'', -N(R'')-(peptide) or R'''' ;

20

Z is H or R'''' ;

R' , R'' , R''' and R'''' are independently H or lower straight or branched chain or cyclic alkyl;

25

n is 0, 1 or 2;

and

where B is -NHR'' or -N(R'')-(peptide), X is SH, and n is 1 or 2;

30

where X is -NHR'' or -N(R'')-(peptide), B is SH, and n is 1 or 2;

where B is H or R'''' , A is HOOC, H_2NOC , (peptide)-NHOC, (peptide)-OOC, X is SH, and n is 0 or 1;

35

where A is H or R'''' , then where B is SH, X is -NHR'' or -N(R'')-(peptide) and where X is SH, B is -NHR'' or -N(R'')-(peptide);

where X is H or R'''' , A is HOOC, H_2NOC , (peptide)-NHOC, (peptide)-OOC and B is SH;

40

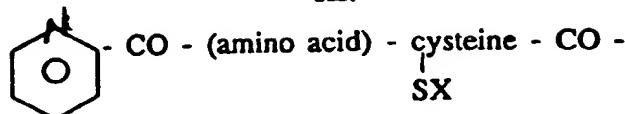
where Z is methyl, X is methyl, A is HOOC, H_2NOC , (peptide)-NHOC, (peptide)-OOC, B is SH and n is 0;

where B is SH and X is SH, n is not 0;

28

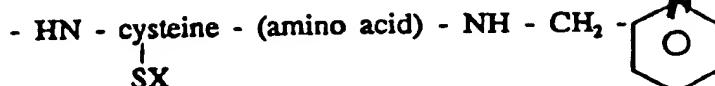
and wherein the thiol moiety is in the reduced form;

III.



5

IV.



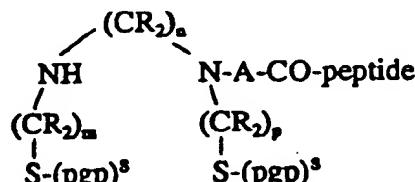
wherein

X = H or a protecting group;

(amino acid) = any amino acid;

10

V.



15

wherein

each R is independently H, CH₃ or C₂H₅;

each (pgp)^s is independently a thiol protecting group or H;

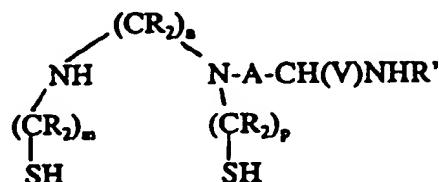
m, n and p are independently 2 or 3;

A = linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof;

20

or

VI.



25

wherein

each R is independently H, CH₃ or C₂H₅;

m, n and p are independently 2 or 3;

A = linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof;

30

V = H or -CO-peptide;

R' = H or peptide;

and wherein when V = H, R' = peptide and when R' = H, V = -CO-peptide;

wherein each R is independently H, lower alkyl having 1 to 6 carbon atoms, phenyl, or phenyl substituted with lower alkyl or lower alkoxy, and wherein
5 each n is independently 1 or 2;

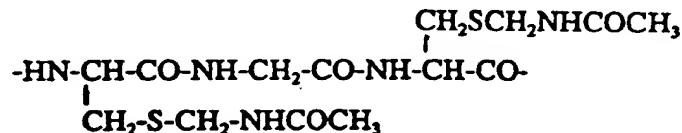
wherein the technetium-99m binding moiety forms a complex with technetium-99m.

4. The reagent of Claim 3 that is radiolabeled with technetium-99m.

5. The reagent of Claim 3 wherein the cysteine of the technetium-
10 99m binding moiety having formula I has a protecting group of the formula
-CH₂-NH-CO-R

wherein R is a lower alkyl having 1 to 6 carbon atoms, 2-,3-,4-pyridyl, phenyl, or phenyl substituted with lower alkyl, hydroxy, lower alkoxy, carboxy, or lower alkoxy carbonyl.

15 6. The reagent of Claim 3 wherein the technetium-99m binding moiety C(ppg)³-(aa)-C(ppg)³ has the formula:



20 7. A complex formed by reacting the reagent of Claim 3 with technetium-99m in the presence of a reducing agent.

8. The complex of Claim 7, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

25 9. A complex formed by labeling the reagent of Claim 3 with technetium-99m by ligand exchange of a prereduced technetium-99m complex.

10. A kit for preparing a radiopharmaceutical preparation, said kit comprising a sealed vial containing a predetermined quantity of the reagent of Claim 3 and a sufficient amount of reducing agent to label the reagent with technetium-99m.

30 11. A method for labeling a reagent according to Claim 3 comprising reacting the reagent with technetium-99m in the presence of a reducing agent.

12. The method of Claim 11, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

13. A method for imaging a site within a mammalian body comprising administering an effective diagnostic amount of the reagent of Claim 4 and detecting the technetium-99m localized at the site in the mammalian body.

5 14. The reagent according to Claim 3 wherein the somatostatin receptor-binding peptide is chemically synthesized *in vitro*.

10 15. The reagent according to Claim 14 wherein the somatostatin receptor-binding peptide is synthesized by solid phase peptide synthesis.

16. The reagent according to Claim 14 wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during *in vitro* chemical synthesis.

15 17. The reagent according to Claim 16 wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during solid phase peptide synthesis.

18. The somatostatin receptor-binding peptide of Claim 1 wherein A¹ is phenylalanine or tyrosine, A² is tryptophan, A³ is lysine and A⁴ is threonine or valine.

20 19. A composition of matter comprising the somatostatin receptor-binding peptide of Claim 1 is selected from the group consisting of peptides having the formula:

25 CH.CO.YW_DKTCTC_{Acm}GC_{Acm}.amide

CH.CO.YW_DKTC

CH.CO.YW_DKTC.amide

30 CH.CO.YW_DKTCT

CH.CO.YW_DKTCT(CH₃OH)

CH.CO.YW_DKTCTGGC_{Mab}.amide

35 CH.CO.FFW_DKTFC

CH₂CO.FFW_DKTFC.amide
5 CH₂CO.FW_DKTC
CH₂CO.FW_DKT.Hcy
10 CH₂CO.FW_DKT.Hcy.amide
 CH₂CO.FW_DKT.Pen
15 CH₂CO.NFFW_DKTFTC
 CH₂CO.FFW_DKTFCC_{Acm}GC_{Acm}.amide
 CH₂CO.FFW_DKTF.Hcy
20 PhCH₂CHCO.YW_DKTC
 CH₂CO.YW_DKT.Hhc
25 CH₂CO.YW_DKT.Hhc.amide
 CH₂CO.FFW_DKTF.Hhc
 CH₂CO.FYW_DKTFC
30 or
 CH₂CO.YW_DKTC
35 20. A composition of matter comprising the reagent of Claim 3 is
 selected from the group consisting of reagents having the formula:
 CH₂CO.YW_DKTCTC_{Acm}GC_{Acm}.amide
 CH₂CO.YW_DKTC
40 CH₂CO.YW_DKTC.amide
 CH₂CO.YW_DKTCT
 CH₂CO.YW_DKTCT(CH₂OH)
45 CH₂CO.YW_DKTCTGGC_{Mab}.amide

CH₂CO.FFW_DKTFC

CH₂CO.FFW_DKTFC.[BAM]

5 CH₂CO.FFW_DKTFC.amide

CH₂CO.FW_DKT.Hcy

10 CH₂CO.FW_DKT.Hcy.amide

CH₂CO.FW_DKT.Pen

CH₂CO.NFFW_DKTFTC

15 CH₂CO.FFW_DKTFCC_{Acm}GC_{Acm}.amide

CH₂CO.FFW_DKTF.Hcy

20 P_nCH₂CHCO.YW_DKTC

CH₂CO.YW_DKT.Hhc

CH₂CO.YW_DKT.Hhc.amide

25 CH₂CO.FFW_DKTF.Hhc

CH₂CO.FYW_DKTFC

30 CH₂CO.FW_DKTC_D

or

CH₂CO.YW_DKTC

35 21. A method for alleviating a somatostatin-related disease in an animal comprising administering a therapeutically effective amount of the composition of Claim 2 to the animal.

22. The method of Claim 21 wherein the animal is a human.

40 23. The method of Claim 21 wherein the therapeutically effective amount of the composition administered to the animal is from about 0.1 to about 49 mg/kg body weight/day.

24. A reagent for preparing a radiolabeled somatostatin receptor-

binding agent comprising the somatostatin receptor-binding peptide of Claim 1 and a radiolabel-binding moiety covalently linked thereto.

25. The reagent of Claim 24 that is radiolabeled with ^{186}Re or ^{188}Re .
26. A complex formed by reacting the reagent of Claim 24 with ^{186}Re or ^{188}Re in the presence of a reducing agent.
5
27. The complex of Claim 26, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.
28. A kit for preparing a radiopharmaceutical preparation, said kit comprising a sealed vial containing a predetermined quantity of the reagent of
10 Claim 24 and a sufficient amount of reducing agent to label the reagent with ^{186}Re or ^{188}Re .
29. The reagent according to Claim 24 wherein the somatostatin receptor-binding peptide is chemically synthesized *in vitro*.
30. The reagent according to Claim 29 wherein the somatostatin receptor-binding peptide is synthesized by solid phase peptide synthesis.
15
31. The reagent according to Claim 29 wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during *in vitro* chemical synthesis.
32. The reagent according to Claim 31 wherein the radiolabel-binding
20 moiety is covalently linked to the somatostatin receptor-binding peptide during solid phase peptide synthesis.
33. A pharmaceutical composition comprising the radiolabeled somatostatin receptor-binding peptide of Claim 25 in a pharmaceutically acceptable carrier.
- 25 34. A method for alleviating a somatostatin-related disease in an animal comprising administering a therapeutically effective amount of the composition of Claim 33 to the animal.
35. The method of Claim 34 wherein the animal is a human.
36. The method of Claim 34 wherein the therapeutically effective
30 amount of the composition administered to the animal is from about 10 to 200 milliCuries of the radiolabeled composition.

37. The reagent of Claim 24 that is radiolabeled with technetium-99m.

38. A pharmaceutical composition comprising the radiolabeled somatostatin receptor-binding peptide of Claim 37 in a pharmaceutically acceptable carrier.

39. A complex formed by reacting the reagent of Claim 24 with technetium-99m in the presence of a reducing agent.

40. The complex of Claim 39, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

41. A kit for preparing a radiopharmaceutical preparation, said kit comprising a sealed vial containing a predetermined quantity of the reagent of Claim 24 and a sufficient amount of reducing agent to label the reagent with technetium-99m.

42. A method for imaging a site within a mammalian body comprising administering an effective diagnostic amount of the reagent of Claim 37 and detecting the technetium-99m localized at the site within the mammalian body.

43. The somatostatin receptor-binding peptide of Claim 1 wherein the reagent further comprises a polyvalent linking moiety covalently linked to a multiplicity of specific binding compounds and also covalently linked to a multiplicity of radiolabel-binding moieties to comprise a reagent for preparing a multimeric polyvalent scintigraphic imaging agent, wherein the molecular weight of the multimeric polyvalent scintigraphic imaging agent is less than about 20,000 daltons.

44. The somatostatin receptor-binding peptide of Claim 43 wherein the polyvalent linking moiety is *bis*-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, *N*-[2-(*N,N'*-*bis*(2-succinimidooethyl)aminoethyl)]-*N⁶,N⁹*-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, *tris*(succinimidyl-ethyl)amine or a derivative thereof.

45. The reagent of Claim 3 for preparing a scintigraphic imaging agent for imaging sites within a mammalian body wherein the reagent further

comprises a polyvalent linking moiety covalently linked to a multiplicity of specific binding compounds and also covalently linked to a multiplicity of radiolabel-binding moieties to comprise a reagent for preparing a multimeric polyvalent scintigraphic imaging agent, wherein the molecular weight of the 5 multimeric polyvalent scintigraphic imaging agent is less than about 20,000 daltons.

46. The reagent of Claim 45 wherein the polyvalent linking moiety is *bis*-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, *N*-[2-(*N,N'*-*bis*(2-succinimidooethyl)aminoethyl]-*N⁶,N⁹-bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, *tris*(succinimidylethyl)amine or a derivative thereof.

10 47. The reagent of Claim 39 wherein the complex of the radiolabel-binding moiety and technetium-99m is electrically neutral.

48. The composition of matter of Claim 20 that is radiolabeled with technetium-99m.

15 49. The composition of matter of Claim 20 that is radiolabeled with ¹⁸⁶Re or ¹⁸⁸Re.